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## Research note

## SARS-CoV-2 infection: the environmental endurance of the virus can be influenced by the increase of temperature

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## ABSTRACT

**Objectives:** To evaluate whether the increase of temperature can influence the environmental endurance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

**Methods:** Virus was inoculated on a plastic surface and harvested at predefined time-points in parallel at 20°C–25°C (room temperature; RT) and at 28°C (June temperature; JT). Samples were tested by TCID<sub>50</sub> titres on Vero cells.

**Results:** Our results confirm that fomite transmission of the emerging SARS-CoV-2 is possible: the virus reserved its ability to infect cells for up to 84 hours at both RT and JT on a plastic surface, with TCID<sub>50</sub> viral titres of 0.67 and 0.25 log<sub>10</sub>, respectively. At RT, an important reduction in the viral titre, from 4 log<sub>10</sub> to 3 log<sub>10</sub> TCID<sub>50</sub>, was observed during the first 24–36 hours. At JT, the same decay was observed more rapidly (between 8 and 12 hours). The rate of viral inactivation by D-value was 24.74 hours at RT and 12.21 hours at JT.

**Conclusions:** This remarkable difference between the two temperatures suggests that virus vitality can be influenced by the environmental temperature and that the hot season could reduce the probability of COVID-19 transmission. **Fabio Magurano, Clin Microbiol Infect 2021;27:289.e5–289.e7**

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## Introduction

The coronavirus disease 2019 (COVID-19) pandemic has been caused by the enveloped betacoronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), transmitted from person to person through respiratory droplets and direct contact, and potentially by indirect contact through fomites [1].

Several studies have shown that viral spread could be influenced by climatic conditions as enveloped viruses tend to reduce their circulation in summer because of high temperature and solar radiation [2,3]. Indeed, the current spread of COVID-19 along the equator and tropics was shown to be significantly less [4], leading to the hypothesis that increase of temperature will influence the environmental endurance of SARS-CoV-2.

In an attempt to predict the epidemic dynamic of COVID-19 during the summer months, we tested the environmental stability of SARS-CoV-2 in parallel at room temperature (RT, 20°C–25°C) and at an average maximum temperature for June (JT) estimated at 28°C in Italy.

## Methods

The strain *BetaCov/Italy/CDG1/2020|EPI ISL 412973|2020-02-20* [5] was used to test the stability of SARS-CoV-2 on a plastic surface (polypropylene). This strain had an initial viral titre of 10<sup>6.8</sup> TCID<sub>50</sub>/mL, a comparable viral load to symptomatic, asymptomatic and minimally symptomatic patients [6].

The viral preparation was spotted in droplets of 10 µL on 24-well plates and allowed to dry for 30 minutes. Then, plates were incubated at both RT and JT for 7 days at relative humidity of 35%–45% (in an air-conditioned room). The virus was harvested at 0, 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 hours by adding 1 mL/well of Eagle's minimal essential medium (EMEM) + 2% fetal calf serum (FCS) and was quantified by end-point titration on Vero cells by TCID<sub>50</sub>

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(dilution of virus at which 50% of the cell cultures are infected): 96-well plates were inoculated with two-fold dilutions of each viral sample (100  $\mu$ L), and 22 000 cells/well in EMEM + 2% FCS were added (100  $\mu$ L). Plates were left in the incubator at 37°C with 5% CO<sub>2</sub> for 6 days and were checked daily to observe the cytopathic effect. The 50% end-point titres were determined using the Spearman–Karber method. Both experiments were conducted in three independent replicates and the limit of detection by the end-point technique was established as 1 log<sub>10</sub>. Ethical approval was not needed for this study.

Viral titres of the daily collections at 0, 24, 48 and 72 hours at RT were also determined by plaque assay in Vero E6 cells. Briefly, 12-well plates were plated with Vero E6 cells (150 000/well in EMEM + 10% FCS) and inoculated with logarithmic dilutions of each sample. Plates were incubated for 1 hour at 37°C, and 4 mL/well of a medium containing 2% Gum Tragacanth + EMEM + 2.5% FCS was added. After 5 days at 37°C with 5% CO<sub>2</sub>, titres were calculated using crystal violet dye in plaque-forming units per milliliter. All the experimental procedures were conducted under biosafety level 3 conditions.

To approximate a normal distribution, the viral titre of each well was log-transformed. Using standard deviation with a 95% confidence interval, the results mostly followed a normal distribution of a set value. The ratio of the standard deviation to the mean was investigated by calculating the coefficient of variance.

## Results

Analysis of data obtained by TCID<sub>50</sub> titration showed that an important reduction in the viral titre, from 4 log<sub>10</sub> to 3 log<sub>10</sub> TCID<sub>50</sub>/mL of medium, was observed during the first 24–36 hours at RT (Fig. 1) with a D-value of 24.74 hours. This trend was confirmed by titration by plaque assay (see [Supplementary material, Figs S1–S4 and Table S1](#)).

At JT, the same decay was observed more rapidly (between 8 and 12 hours), indicating that viral infectivity can be influenced by higher temperature with a D-value of 12.21 hours.

This decay trend continued until 84 hours, showing a remarkable difference between the two temperatures. In both the experimental conditions, the virus was no longer detectable at 96 hours.

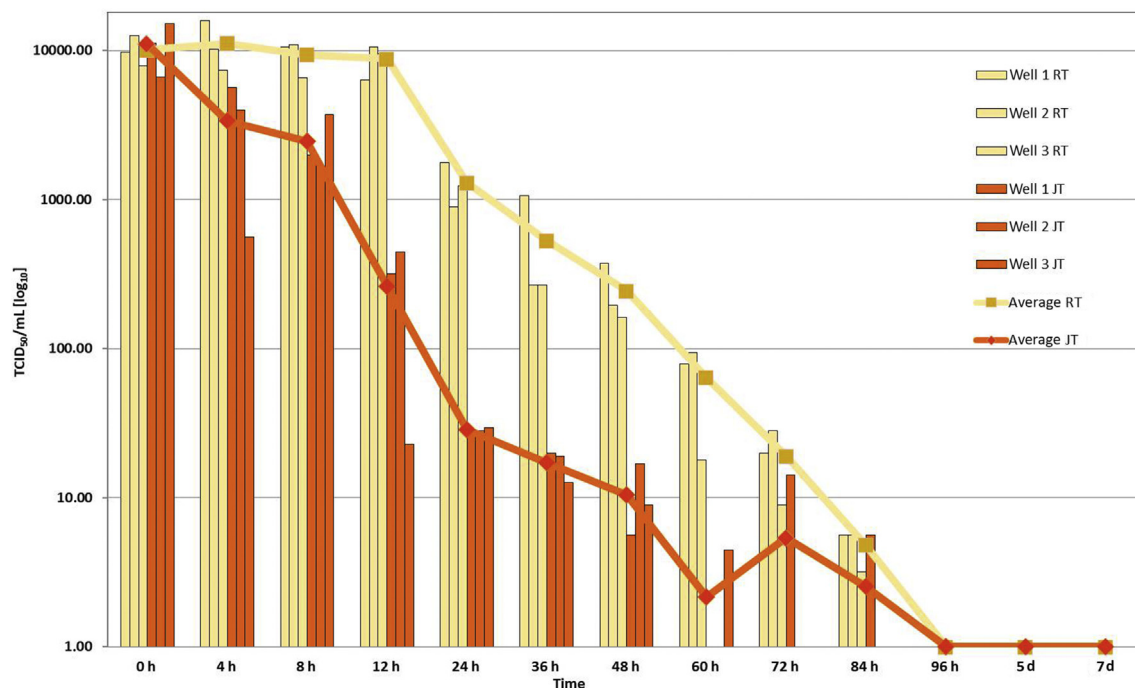
## Discussion

The present study confirms that fomite transmission of the emerging SARS-CoV-2 is possible [7], because the virus remained viable on plastic surfaces for up to 84 hours at both RT and JT. In both experimental conditions, the virus was not detectable at 96 hours. End-point titration showed that the virus infectivity on a plastic surface rapidly declined during the first 24–36 hours at RT and there was a remarkable difference between the two temperatures.

Contaminated surfaces are reported to be significant vectors in the transmission of infections and survival of viruses on a variety of fomites has been demonstrated for other coronaviruses, influenza viruses, paramyxoviruses, poxviruses and retroviruses [8–10].

The results of this study support the hypothesis that during the hot season the increase of temperature may influence the environmental endurance of SARS-CoV-2 and reduce the probability of virus transmission. The main limitation of this study is the small number of temperatures tested, which does not allow accurate evaluation of the influence of environmental temperature on virus stability. Moreover, we cannot establish at what times and temperatures viral transmission is no longer possible because we do not know the minimum infectious dose of SARS-CoV-2.

In conclusion, the increase of temperature observed in summer may influence the environmental endurance of SARS-CoV-2 but this does not influence the need to maintain social distancing measures.



**Fig. 1.** Relation between viral titre (determined by 50% end-point titration; TCID<sub>50</sub>) and time of collection in logarithmic scale. Average viral titres in samples collected at either room temperature (RT) or June temperature (JT) are plotted as a function of time-points after infection.

## Transparency declaration

The authors declare no competing interests.

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No external funding was received.

## Authors' contributions

MF conceived and designed the study; MF, BM and MA performed the experiments; MF, BM and NL analysed the data; MF and BM wrote the manuscript, and NL and RG critically revised the manuscript.

## COVID-19 study group

In addition to the authors, the COVID-19 Study Group consisted of: Eleonora Benedetti, Concetta Fabiani, Stefano Fiore, Maedeh Kojouri, Paola Bucci, Emilio D'Ugo and Silvia Gioacchini.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2020.10.034>.

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